Claims

1. (currently amended) A ~~device~~ test strip for determining presence and/or amount of an analyte in a ~~fluid~~ liquid sample comprising:

a mobilization zone ~~comprising a mobile or mobilizable detectable tracer molecule~~;

a mobile or mobilizable detectable tracer in the mobilization zone;

a sample application area;

a primary capture area comprising a first immobilized binding partner having a binding affinity for the analyte and a binding affinity for the detectable tracer ~~molecule~~; and

a secondary capture area comprising a second immobilized binding partner having a ~~binding affinity for the analyte and a~~ binding affinity for the detectable tracer ~~molecule~~,

wherein the sample application area, mobilization zone, primary capture area and secondary capture area are in ~~fluid continuous contact, and the first immobilized binding partner has an equal or a lower apparent affinity for the analyte than it has for the detectable tracer molecule~~ a path of liquid flow along a bibulous substrate from the sample application area distally through the mobilization zone to the primary capture area and then to the secondary capture area, wherein the detectable tracer is present on the test strip in a position that a distal flow of analyte reaches the primary capture area before a distal flow of tracer reaches the primary capture area such that subsequent binding of detectable tracer to first immobilized binding partner is inhibited and unbound detectable tracer continues along the path of flow distally to the second immobilized binding partner to provide a signal from the secondary capture area that indicates the presence of the analyte in the liquid sample.

2. (currently amended) The ~~device~~ test strip of claim 1, wherein the detectable tracer ~~molecule is associated with the device in such a way that, during operation of the device, it contacts~~ is positioned within the test strip in a position that the distal flow of tracer reaches the primary capture area after ~~a sample contacts~~ the distal flow of analyte reaches the primary capture area.

3. (currently amended) The ~~device~~ test strip of claim 1, wherein~~, during operation of the device,~~ the detectable tracer is heavier than the analyte and therefore ~~molecule~~ migrates

through the ~~device~~ test strip at a rate slower than a rate at which the analyte in ~~a~~ the liquid sample migrates through the ~~device~~ test strip.

4. (currently amended) The test strip ~~device~~ of claim 3, wherein the detectable tracer is selected to interact with the test strip to slow ~~slower~~ migration of the tracer relative to migration of the analyte~~molecule is caused by a molecular weight of the tracer molecule~~ .

5. (currently amended) The ~~device~~ test strip of claim ~~3~~2, wherein the test strip is a bibulous porous strip and ~~slower migration of~~ the detectable tracer ~~molecule is caused by a physical or temporal placement of the tracer molecule on the device~~ is larger than the analyte and therefore migrates along the path of liquid flow through the bibulous porous strip more slowly than the analyte migrates along the path of liquid flow.

6. (currently amended) The ~~device~~ test strip of claim 5, wherein the detectable tracer ~~molecule~~ is positioned beneath the surface of the test strip on which the liquid ~~placed on the device after a~~ sample is placed ~~on the device~~ such that the detectable tracer migrates through the test strip to the primary capture area more slowly than analyte in the liquid sample.

7. (currently amended) The ~~device~~ test strip of claim ~~1, further comprising at least one filter pad in a path of flow of the fluid~~ 4, wherein the tracer is selected based on its polarity or charge to provide specific migration characteristics that retard migration of the tracer relative to migration of the analyte.

8. (currently amended) The ~~device~~ test strip of claim ~~5~~ 7, wherein the ~~filter pad~~ test strip is pretreated with at least one reagent to enhance the sensitivity of the assay device by delaying migration of the tracer relative to the analyte.

9. (currently amended) The ~~device~~ test strip of claim ~~6~~ 8, wherein the at least one reagent is selected from the group consisting of ~~buffers, detergents, and anticoagulants~~ sucrose, mannitol, glycerol, polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), and mixtures thereof.

10. (currently amended) The ~~device~~ test strip of claim 1, wherein the first and second immobilized binding partners are ~~selected from the group consisting of~~ antibodies, antigens, ~~and~~ haptens, lectins or receptors.

11. (currently amended) The ~~device~~ test strip of claim 1, wherein the first and second immobilized binding ~~agents~~ partners for the analyte are identical.

12. (currently amended) The ~~device~~ test strip of claim 1, wherein the first and second immobilized binding ~~agents~~ partners are each ~~anti-analyte~~ antibodies, and the detectable tracer comprises an analyte analog, and the first binding partner is an antibody having a greater affinity for the analyte than the analyte analog.

13. (currently amended) The ~~device~~ test strip of claim 1, wherein the detectable tracer ~~molecule~~ comprises ~~an analyte molecule or~~ an analyte analog ~~molecule~~.

14. (currently amended) The ~~device~~ test strip of claim 1, wherein the detectable tracer ~~molecule~~ comprises a visually detectable label covalently attached to analyte or an analyte analog.

15. (currently amended) The ~~device~~ test strip of claim 1, wherein the dectable tracer comprises a detectable tracer for an analyte ~~is~~ selected from the group consisting of an ~~antigens~~ antigen of an infectious ~~diseases~~ disease, an ~~antibodies to antigens~~ antigen to an antibody of an infections ~~diseases~~ disease, a ~~hormones~~ hormone, a growth ~~factors~~ factor, a therapeutic ~~drugs~~ drug, a ~~drugs~~ drug of abuse, ~~and products~~ a product of the metabolism of ~~drugs~~ a drug of abuse, and ~~haptens~~ a hapten.

16. (currently amended) The ~~device~~ test strip of claim 15, wherein the detectable tracer comprises a detectable tracer for an analyte comprising ~~antibodies are~~ an antibody selected from the group consisting of ~~antibodies~~ an antibody to ~~HIV~~ Human Immunodeficiency Virus (HIV), ~~antibodies~~ an antibody to ~~HTLV~~ Human T-Cell Lymphotropic Virus (HTLV), ~~antibodies~~

an antibody to *Helicobacter pylori*, ~~antibodies~~ an antibody to hepatitis, ~~antibodies~~ an antibody to measles, ~~antibodies~~ an antibody to mumps, and ~~antibodies~~ an antibody to rubella.

17. (currently amended) The ~~device~~ test strip of claim 15, wherein the detectable tracer comprises a detectable tracer for an analyte comprising a therapeutic ~~drugs and drugs~~ drug or drug of abuse or products of the metabolism of ~~drugs~~ a drug of abuse, ~~are~~ wherein the analyte is selected from the group consisting of tetrahydrocannabinol, nicotine, cotinine, ethanol, theophylline, phenytoin, acetaminophen, lithium, diazepam, nortryptyline, secobarbital, and phenobarbitol, methamphetamine and fragments, mimetics, and analogs or derivatives thereof.

18. (currently amended) The ~~device~~ test strip of claim 17, wherein the detectable tracer comprises a detectable tracer conjugate for an analyte that is a product of metabolism of a drug of abuse, and the product of metabolism comprises cotinine.

19. (currently amended) The ~~device~~ test strip of claim 15, wherein the detectable tracer comprises a detectable tracer conjugate for an analyte comprising a hormone, and the hormone is ~~hormones are~~ selected from the group consisting of testosterone, estradiol, estriol, 17-hydroxyprogesterone, progesterone, thyroxine, thyroid stimulating hormone, follicle stimulating hormone, and luteinizing hormone, and fragments, mimetics, analogs or derivatives thereof.

20. (currently amended) The ~~device~~ test strip of claim 1, wherein the quantity of the second specific binding partner in the secondary capture area is such that the quantity of detectable tracer ~~molecule~~ binding to the secondary capture area, and by correlation the amount of the analyte in ~~a tested~~ the liquid sample, is indicated by ~~the~~ intensity of a detection signal of the detectable tracer ~~molecule~~ in the secondary capture area.

21. (currently amended) The ~~device~~ test strip of claim 1, wherein the ~~area of the~~ secondary ~~specific~~ binding area of the test strip is divided into at least two discrete and non-overlapping bands, ~~partner immobilized on the chromatographic medium is divided into at least two discrete and non-overlapping bands,~~ with ~~the~~ a quantity of the second specific binding

partner in each band being such that the quantity of tracer ~~molecule~~ binding to the secondary capture area, and by correlation the amount of the analyte in a tested sample, is indicated by the number of bands to which the tracer molecule binds.

22. (currently amended) A ~~device~~ bibulous test strip for determining presence and/or amount of an analyte in a ~~fluid~~ liquid sample comprising:

a mobilization zone comprising a ~~mobile or~~ mobilizable detectable tracer, wherein the detectable tracer comprises an analyte or analyte analog covalently coupled to a detectable label, and the detectable tracer has been selected to have a slower rate of migration of the tracer than the analyte through the bibulous test strip ~~molecule~~;

a sample application area on the test strip;

a primary capture area comprising a first immobilized ~~binding partner~~ antibody having a binding affinity for the analyte and a binding affinity for the detectable tracer ~~molecule~~; and

a secondary capture area comprising a second ~~immobilized binding partner~~ antibody having a binding affinity for the analyte and a binding affinity for the detectable tracer molecule,

wherein, the sample application area, mobilization zone, primary capture area and secondary capture area are in a path of liquid flow from the sample application area distally through the mobilization zone to the primary capture area and then to the secondary capture area, ~~during operation of the device,~~ such that a liquid flow of the analyte reaches the primary capture area before the tracer such that subsequent binding of tracer to the first antibody is inhibited and unbound tracer continues along the path of flow to the second antibody to provide a signal from the secondary capture area that indicates presence of the analyte in the liquid sample ~~the detectable tracer molecule contacts the primary capture area after the sample contacts the primary capture area~~.

23. (currently amended) ~~A method for detecting and/or quantitating an analyte in a fluid sample~~The test strip of claim 22 wherein the tracer comprises the analyte or analyte analog covalently linked to the detectable label by a linker molecule~~, comprising:~~

~~applying a liquid sample to a substrate along which the samples migrates sequentially to a primary capture area and a secondary capture area, wherein the primary capture area binds the analyte with an equal or a lower apparent affinity than it binds a detectable tracer molecule; and the secondary capture area binds the detectable tracer molecule with high affinity; and~~

~~reading a detectable signal from bound detectable tracer molecule in the secondary capture area, wherein the detectable signal indicates the presence of analyte in the sample~~.

24. (currently amended) The ~~method~~ test strip of claim ~~23~~22, wherein the, ~~further comprising:~~

~~applying a~~ detectable tracer ~~molecule to the substrate~~ is selected such that a diameter of the tracer is small enough to migrate through the bibulous test strip but not so large that it is trapped by pores of the bibulous test strip.

25. (currently amended) The ~~method~~ test strip of claim 24, wherein the detectable label comprises a latex particle having a diameter of 30-400 nm ~~tracer molecule is applied to the substrate before the sample~~.

26. (currently amended) The ~~method~~ test strip of claim 24, wherein the detectable ~~tracer molecule is applied to the substrate after the sample~~ label comprises a colloidal gold particle.

27. (currently amended) The ~~method~~ test strip of claim ~~24~~23, wherein ~~the detectable tracer molecule and the sample are applied simultaneously~~ a linker molecule forms a stable covalent linkage between the detectable label and the analyte or analyte analog.

28. (currently amended) The ~~method~~ test strip of claim 23, wherein the ~~detectable signal has an intensity, and the intensity of the signal correlates with the amount of analyte in the sample~~ linker molecule comprises from 1-20 carbons and 0-10 heteroatoms.

29. (currently amended) The method of claim ~~23~~ 22, wherein the first ~~binding partners are immobilized on the substrate~~ antibody has a greater affinity for the analyte than the analyte analog.

Claim 30 (canceled)

31. (currently amended) A method for detecting and/or quantitating an analyte in a ~~fluid~~ liquid sample, comprising:

contacting the ~~fluid~~ liquid sample with the ~~device~~ sample application area of the test strip of claim 1~~.~~ , and allowing the liquid sample to mobilize the tracer such that the distal flow of tracer migrates with the liquid sample, but reaches the primary capture zone after distal flow of analyte in the liquid sample;

wherein the distal flow of analyte that reaches the primary capture zone occupies first immobilized binding partner such that subsequent binding of the detectable tracer to the first immobilized binding partner is inhibited, whereby unbound detectable tracer continues along the path of flow distally to bind to the second immobilized binding partner and provide a signal from the secondary capture area that indicates the presence of the analyte in the liquid sample.

Claims 32-34 (canceled)

35. (currently amended) The method of claim 31, further comprising quantifying ~~the~~ an amount of analyte in the liquid sample, wherein the amount of analyte in the liquid sample ~~is proportional to~~ determines an intensity of the signal ~~in the second~~ from the tracer in the secondary capture area.

36. (currently amended) The method of claim 31, wherein the liquid sample migrates along the test strip ~~device~~ in the path of liquid flow by capillary action.

37. (original) The method of claim 31, wherein the analyte has a molecular weight of about 100 – 1,000 Daltons.

38. (original) The method of claim 31, wherein the analyte has a molecular weight of greater than 1,000 Daltons.

Claim 39 (canceled)

40. (currently amended) The method of claim ~~39~~ 31, wherein the ~~fluid~~ liquid sample is selected from the group consisting of urine, blood, tears, sweat and saliva.

41. (currently amended) The method of claim 40, wherein the ~~fluid~~ liquid sample is saliva.

42. (currently amended) The method of claim 41, ~~further comprising providing an oral fluid sample~~ wherein the saliva is combined with a bile acid ~~bile~~ or bile salt in a concentration ~~sufficient to reduce~~ that reduces occurrence of false positives in the immunoassay.

43. (currently amended) The method of claim 42, wherein the bile acid or bile salt ranges in concentration from about 0.1 weight percent to about 1.0 weight percent of the ~~oral fluid~~ saliva/bile salt or saliva/bile acid combination.

44. (currently amended) The method of claim 43, further comprising contacting a chelator of divalent cations with the ~~oral fluid~~ saliva sample.

45. (currently amended) A test kit for the detection and/or the determination of an analyte in a sample comprising:

(a) ~~the chromatographic assay device~~ the test strip of claim 1; and

(b) instructions for using the test strip such that the flow of analyte in the liquid sample reaches the primary capture zone before the flow of tracer.

Claim 46 (canceled)

47. (new) The method of claim 31, wherein the detectable tracer is contained beneath an external surface of the test strip, and the liquid sample is applied to the external surface of the test strip, such that the flow of tracer migrates at a slower rate along the path of flow than flow of analyte in the liquid sample migrates along the path of flow toward the primary capture area.

48. (new) The method of claim 31, wherein the liquid sample is applied to the test strip at a position closer to the primary capture area than the detectable tracer is applied to the test strip.

49. (new) The method of claim 31, wherein the detectable tracer interacts with the test strip to slow its flow along the path of liquid flow more than flow of analyte in the liquid sample is slowed such that any analyte in the liquid sample reaches the primary capture zone ahead of the detectable tracer.

50. (new) A method for making a test strip for detecting and/or quantitating an analyte in a liquid sample, comprising:

providing a bibulous test strip that defines a path of liquid flow from a sample application area distally through a mobilization zone to a primary capture area to a secondary capture area;

providing in the primary capture area a first immobilized binding partner having a binding affinity for the analyte, and providing in the secondary capture area a second immobilized binding partner having a binding affinity for the analyte;

providing in the mobilization zone a complex comprising a detectable tracer coupled to analyte or an analyte analog, wherein the complex has been selected to migrate more slowly through the bibulous test strip than the analyte;

wherein when the test strip is contacted with the liquid sample in the sample application area of the test strip of claim 1, the liquid sample mobilizes the detectable tracer such that the detectable tracer migrates behind the liquid sample, such that the tracer reaches the primary capture zone after analyte flow in the liquid sample reaches the primary capture area, and the analyte flow that reaches the primary capture zone occupies first immobilized binding partner such that subsequent binding of detectable tracer to first immobilized binding partner is inhibited, whereby unbound detectable tracer continues along the path of liquid flow distally to

bind to second immobilized binding partner and provide a signal from the secondary capture area that indicates the presence of the analyte in the liquid sample.

51. (new) The method of claim 50, wherein the detectable tracer comprises an analyte or analyte analog covalently linked to a detectable molecule by a linker.

52. (new) The method of claim 51, wherein the linker is selected to increase the weight of the detectable tracer.

53. (new) The method of claim 52, wherein the linker comprises bovine serum albumin (BSA).

54. (new) The method of claim 49, wherein the analyte and detectable tracer have chemical characteristics that provide differential rates of migration of the analyte and detectable tracer along the path of liquid flow to the primary capture area.

55. (new) The method of claim 54, wherein the different chemical characteristics comprise size, polarity or charge.

56. (new) The test strip of claim 1, wherein the sample application area overlaps the mobilization zone.

57. (new) The test strip of claim 1, wherein the sample application area does not overlap the mobilization zone.

58. (new) The test strip of claim 57, wherein the mobilization zone is distal in the path of flow to the sample application area.

59. (new) The test strip of claim 50, wherein the first and second immobilized binding partner for the analyte are identical.

60. (new) The test strip of claim 50, wherein the first and second immobilized binding partner are each anti-analyte antibodies, and the first immobilized binding partner has a greater affinity for the analyte than the complex.

61. (new) The test strip of claim 50, wherein the detectable tracer is a tracer conjugate comprising an analyte or an analyte analog.

62. (new) The test strip of claim 50, wherein the detectable tracer comprises a visually detectable label.

63. (new) A device for detecting an analyte in a liquid, comprising:

a porous test strip that defines a path of liquid flow from a proximal sample application area toward a distal end of the test strip, the test strip comprising sequentially from the proximal sample application area:

(a) a mobilization zone comprising a conjugate;

(b) a primary capture area comprising binding sites that have a binding affinity for the analyte and the detectable conjugate; and

(c) a secondary capture area comprising binding sites that have a binding affinity for the detectable conjugate;

wherein the detectable conjugate flows more slowly through the porous test strip than the analyte flows through the test strip after liquid to be analyzed is applied to the proximal sample application area, such that binding sites in the primary capture area are already at least partially occupied by analyte when the detectable conjugate flow reaches the primary capture area, such that distal flow of detectable conjugate continues through the primary capture area to the secondary capture area where the detectable conjugate binds to the binding sites of the secondary capture area and is detectable to indicate the presence of analyte in the liquid.

64. (new) The test strip of claim 63, wherein the primary capture area comprises binding partners immobilized on the test strip, and the secondary capture area comprises binding partners immobilized on the test strip, and the binding partners are antibodies that bind both the analyte and the detectable conjugate.

65. (new) The test strip of claim 63, wherein the conjugate comprises an analyte or analyte analog covalently bound to a detectable label by a linker molecule that slows flow of the conjugate through the test strip relative to flow of the analyte through the test strip.

66. (new) The test strip of claim 65, wherein the detectable conjugate comprises analyte analogs separately conjugated to a detectable label.

67. (new) The test strip of claim 63, wherein the detectable conjugate is positioned in a position in the test strip, or interacts with the porous test strip, to flow more slowly through the porous test strip than the analyte flows through the porous test strip.

68. (new) The test strip of claim 63, wherein the sample application area is more superficially positioned on the test strip than the conjugate, such that sample applied to the test strip encounters less resistance to capillary flow and flows more quickly through the test strip than the conjugate flows through the test strip.

69. (new) The test strip of claim 63, wherein the detectable conjugate is selected to be heavier than the analyte so that the detectable conjugate flows more slowly through the porous test strip than the analyte flows through the test strip.

70. (new) The test strip of claim 65, wherein the linker molecule comprises bovine serum albumin.

71. (new) The test strip of claim 70, wherein the detectable conjugate comprises an analyte analog and a tracer molecule linked by the linker molecule.

72. (new) The test strip of claim 63, wherein the detectable conjugate interacts with the porous test strip though size, polarity or charge of the conjugate to flow more slowly through the test strip than the analyte.

73. (new) The test strip of claim 72, wherein the detectable conjugate comprises a colloidal gold particle of a dimension of at least 30 nm, or a latex particle of a dimension at least 200 nm.

74. (new) The test strip of claim 71, wherein the analyte is smaller in size than the detectable conjugate, and the detectable conjugate is smaller in size than pores of the porous test strip.